New Fuchsin Kit

Reference: AP13102

INTENDED USE AND PRESENTATION:

For research use only.
AP13102. 6 mL / 60 test

SUMMARY, EXPLANATION AND LIMITATIONS:

New Fuchsin Kit is developed for immunohistochemical and in situ-hybridisation staining procedures with alkaline phosphatase (AP). New Fuchsin leads to the formation of a magenta-red precipitate at the location of the target antigen or target nucleic acid. The precipitate is insoluble in aqueous and organic solvents and can be observed by light microscopy or fluorescence microscopy (Texas Red filter).
Immunohistochemistry (IHC) is a complex technique in which immunological and histological detection methods are combined. In general, the manipulation and processing of tissues before immunostaining, especially different types of tissue fixation and embedding, as well as the nature of the tissues themselves may cause inconsistent results (Nadji and Morales, 1983). In some tissues endogenous alkaline phosphatase activity may cause non-specific staining. However, neither intestinal nor placental alkaline phosphatase can be blocked with levamisole. Therefore, tissues of this origin should be stained with peroxidase detection systems (i.e. AP11345). Background staining due to endogenous biotin can be blocked through an avidinbiotin blocking step prior to the primary antibody incubation step. A higher sensitivity can be obtained when a second chromogenic substrate step is used (i. e. 2 x 20 min New Fuchsin). Because of the short stability of the working solution it should be prepared directly before use. Inadequate counterstaining and mounting can influence the interpretation of the results. A longer exposure to absolute ethanol can result in decreasing staining intensity.

APPLICATIONS:

New Fuchsin Kit is intended for immunohistochemical and in situ-hybridisation staining procedures with AP.
The interpretation of the stain results is the full responsibility of the user. Any experimental result must be confirmed by a medically established diagnostic product or procedure.

REAGENT PROVIDED:

0.5 ml	**New Fuchsin Solution** (Chromogen)	Reagent 1
0.5 ml	**New Fuchsin Activator**	Reagent 2
6 ml	**Naphthol-Phosphate Buffer**	Reagent 3
1	**Dilution Vial**	

METHOD AND PROCEDURE:

Principle of the method: The IHC as technique to demonstrate the presence of an antigen in tissues and cells, is a sequential procedure of several steps: the application of antibody specific for the antigen of interest (primary antibody), the detection and visualization of bound antibody by one of a variety of enzyme chromogenic systems and washing steps. The chromogenic enzyme activation results in a visible product at the site where the antigen is located. The results can be evaluated in a light microscope.
Specimen: Formalin-fixed paraffin-embedded tissue section.
Reagent preparation:
1) Mix 50 µl (1 drop) New Fuchsin Solution (Reagent 1) with 50 µl (1 drop) New Fuchsin Activator (Reagent 2) in the provided Dilution Vial and incubate for about 2 - 10 minutes at room temperature.
2) Add 0.9 ml Naphthol-Phosphate Buffer (Reagent 3) and mix thoroughly.
3) The working solution is stable for about 5 - 10 minutes and should therefore be prepared directly before use.
Procedure:
1) Rinse the slide with wash buffer after the previous incubation step.
2) Apply the freshly prepared New Fuchsin working solution onto the slide. Incubate for 20 - 30 minutes.
3) Rinse with distilled H_2O.
4) Counterstain with haematoxylin for about 30 seconds up to 5 minutes (depending on the desired staining intensity).
5) Rinse with distilled H_2O.
6) Blueing in tap water for at least 5 minutes.
7) Dehydrate through a graded series of ethanol and clear in xylene. Mount with a permanent mounting medium.
Note: It is also possible to mount New Fuchsin with aqueous mounting media.
See our web site at www.gennova-europe.com for detailed protocols ancillary reagents and support products.

REQUIRED MATERIALS BUT NOT SUPPLIED:

All reagents, materials, and laboratory equipment for IHC procedures are not provided with this product. This includes adhesive slides and cover slips, positive and negative control tissues, Xylene or adequate substitute, ethanol, distilled H_2O, heat pretreatment equipment (pressure cooker, steamer, microwave), pipettes, Coplin jars, glass jars, moist chamber, histological baths, negative control reagents, counter-staining solution, mounting materials, and microscope.
Buffered solutions for antigen retrieval, enzyme treatments, highly sensitive detection systems, and other auxiliary reagents are available from Gennova Scientific.

STORAGE AND STABILITY:

The solutions should be stored at 2-8 °C without further dilution. Please store the reagents in a dark place and do not freeze them. Under these conditions the solutions are stable up to the expiry date indicated on the label. Do not use product after the expiry date. The working solution is stable for about 5 - 10 minutes and should therefore be used directly after preparation. Excess working solution needs to be disposed as hazardous substance If the product is stored under different conditions from those stipulated in these technical indications, the new conditions must be verified by the user.
Gennova Scientific guarantees that the product will maintain all of the described characteristics from the production date until the expiration date, as long as the product is stored and

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used as recommended. No other guarantees are provided. Under no circumstances is Gennova Scientific obliged to cover damages caused by use of this reagent.

TROUBLESHOOTING:

If unusual staining is observed or any other deviations from the expected results, please read these instructions carefully, along with the instructions from the detection system. If this does not solve the problem, please contact Gennova Scientific's technical support department or your local distributor.

PRECAUTIONS:

Use only by qualified personnel.

Some of the reagents could be hazardous for your health. Wear protective clothing to avoid contact of reagents or specimen with eye, skin or mucous membrane. In case of a reagent or specimen coming into contact with a sensitive area, wash the area with large amounts of water. Microbial contamination of the reagents must be avoided, since otherwise non-specific staining might appear. Sodium azide (NaN_3), used for stabilisation, is not considered hazardous material in the concentrations used. Sodium azide deposits in drainage pipes made of lead or copper can result in the formation of highly explosive metallic azides. To avoid such deposits in drainage pipes, sodium azide should be discarded in a large volume of running water. Material safety data sheets (MSDS) are available upon request.

PERFORMANCE CHARACTERISTICS:

Gennova Scientific has performed studies to evaluate the functioning of the kit for use with standard detection systems, concluding that the product has been found to be suitable for the intended use.

BIBLIOGRAPHY:

Elias JM "Immunohistopathology – A practical Approach to Diagnosis" ASCP Press 2003.

Nadji M, Morales AR. Immunoperoxidase, part 1: the techniques and its pitfall. Lab Med 1983; 14:767-770.

REF	Catalog number	LOT	Batch code	RUO	Research use only
	Temperature limitation		Expiration date	Σ	Test number
	Manufacturer		See instruction for use		

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